

ORIGINAL ARTICLE



Impact of Cocoa Seed Extract Toothpaste on Plaque Reduction in Pediatric Populations

Cut Aja Nuraskin*, Teuku Salfiyadi, Reza Reza and Ainun Mardiah

Department of Dental Health, Politeknik Kesehatan Kemenkes Aceh, Aceh Besar 23231, Indonesia

* Correspondence: cutaja82@yahoo.co.id

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Abstract

Plaque is a soft deposit that forms a helical biofilm layer and attached tightly to the tooth surface, in which its presence requiring the use of specific control agents. The safest and most effective plaque control is to use a toothbrush and toothpaste. On the other hand, extract from *Theobroma cacao* L. seeds contains antioxidant and antibacterial secondary metabolites which could act as a toothpaste active agent. The aim of this research was to determine the impact of *Theobroma cacao* L. seeds-added toothpaste in reducing plaque index of among children. This is a non-blinding clinical experiment with a pre-test and post-test research design. The research subjects were 30 healthy children aged 8-10 years which were randomly divided into three arms (treatment and control). The results revealed that *Theobroma cacao* L. extract used as the active ingredient contained contains phenolics, tannins, flavonoids, terpenoids, saponins, and alkaloids. Significantly higher plaque index reductions were observed among children treated with toothpaste containing 1% (mean difference=2.060; p=0.06) as compared to control. In conclusion, the *Theobroma cacao* L. seeds-added toothpaste could significantly reduce plaque index among healthy children.

Introduction

Dental and oral health have a significant impact on overall body health. Apart from their role in mechanical food digestion, teeth are also important for a person's psychological and social functions [1]. Therefore, maintaining oral and dental hygiene from an early age is crucial. Various organic deposits such as dental plaque, calculus, and white matter can cause various oral health problems such as dental caries and gum disease. Dental plaque is a soft deposit that adheres to the surface of the teeth and gums and is the main cause of dental caries [2]. Dental plaque can be managed through both mechanical and chemical methods. Mechanical methods, such as regular brushing and flossing, are highly effective in controlling plaque buildup [3]. Brushing teeth at least twice a day with fluoride toothpaste aids in removing plaque and preventing dental decay. Additionally, using dental floss is essential for cleaning areas between teeth that are challenging to reach with a toothbrush [4]. In addition to mechanical cleaning, chemical approaches can also be employed to control dental plaque [5,6]. An example of such an approach is mouthwash containing antiseptics or fluoride. This type of mouthwash assists in eradicating the bacteria responsible for plaque formation and reinforces tooth enamel, contributing to overall dental health [7].

Along with technological advancements, innovation is imperative in the formulation of toothpaste, incorporating additional substances that promote dental health while ensuring safety [8–10]. One such addition is herbal ingredients known for their antibacterial properties. Among these, cocoa beans (*Theobroma cacao* L.) stand out and are reported to contain anthocyanins and procyanidins as primary flavonoids. Polyphenols, including flavonoids, exhibit preventive effects against infectious and degenerative oral diseases [11]. A previous study

highlighted the effective antibacterial properties of *T. cacao* seed extract, particularly in inhibiting *Porphyromonas gingivalis* [12]. Moreover, this extract demonstrates potent antioxidant activity, with an IC₅₀ value of 170 ppm in the 2,2-diphenyl-1-picrylhydrazyl assay. *T. cacao* seed extract effectively inhibits bacterial growth across a concentration range of 6.25% to 100% [12]. Based on a preliminary investigation, the methanol extract from *T. cacao* seed demonstrates antibacterial properties that effectively inhibit the growth of dental caries-related bacteria, namely *Streptococcus mutans*.

Dental and oral health challenges persist in Indonesia, with a prevalence rate of 63%. In Aceh Province, 47.0% of its population experience dental problems, where the incidences are concentrated in children aged 5-9 years and older adults ages 55-65 years populations, with prevalence rates of 92.6% and 96.2%, respectively [13]. These figures underscore the ongoing significance of dental and oral health concerns, particularly among school-aged children. An initial survey conducted at State Elementary School Negeri 1 (SDN 1) Pagar Air identified persistent high levels of dental plaque despite previous research efforts. The aim of this study is to assess the effectiveness of *T. cacao* seeds-added toothpaste in reducing the plaque index among students attending SDN 1 Pagar Air, Aceh Besar District, Aceh Province, Indonesia.

Materials and Methods

Study Design and Subjects

The study employed a non-blinding clinical experimental research design utilizing a post-test-only control group. The research was conducted from March to June 2023, involving students from SDN 1 Pagar Air, situated in Aceh Besar Regency, Aceh Province, Indonesia. The toothpaste formulation was developed in Politeknik Kesehatan Kemenkes Aceh's pharmacy laboratory. Plaque index assessments were conducted in June 2023. Inclusion criteria included individuals aged 8-10 with healthy teeth. A total of 30 participants were recruited for the study.

Collection and Extraction of T. cacao Seeds

A total of 2 kg of dry cocoa beans were randomly collected from the Geothermal Area of Sare Aceh village, situated in the Lembah Selawah, Aceh Besar Regency. The extraction process involved macerating 2 kg of ripe yellow cocoa beans, which were air-dried in an open environment devoid of direct sunlight. Subsequently, 1 kg of the dried samples were processed into fine powder and subsequently subjected to maceration with methanol solvent for three cycles, each lasting 24 hours. The resultant filtrate was evaporated using a rotary evaporator until a concentrated methanol extract was obtained.

Qualitative Phytochemical Test

The qualitative identification of secondary metabolites in the *T. cacao* seeds extract was performed in accordance with a guideline from a previous report.[8] Briefly, to identify the presence of alkaloids, 1 g of dry sample was initially ground, followed by the addition of 1 mL of concentrated ammonia. The mixture was then crushed and filtered. Subsequently, 10 ml of HCl 0.5 N was added and vigorously shaken. The HCl layer was separated and divided into three tubes. The presence of alkaloids was indicated by the formations of white precipitate, reddish precipitate, and brown precipitate in the tube added with Mayer's, Dragendorff's, and Wagner's reagents, respectively. In a different test, a total of 10 g *T. cacao* seeds extract was mixed with distilled water and vigorously shaken until stable foam formation occurred, indicating the presence of saponin compounds. The solution was then hydrolyzed with HCl and subsequently tested with the Liebermann-Burchard reagent. A green or blue formation indicated the presence of steroid saponin compounds, while a red color indicated the presence of triterpenoid saponin compounds. As for the flavonoids, they were identified by observing the appearance of a pink or purple coloration after the subsequent additions of 0.5 g Mg and HCl 0.5 M.

Preparation of Toothpaste Formulation

The preparation of the toothpaste formulation commenced with sterilizing the tools and preparing all necessary materials and equipment. Thereafter, all ingredients in the toothpaste formula were meticulously weighed according to the desired proportions. These ingredients included calcium carbonate, glycerin, sodium carboxymethyl cellulose (Na-CMC), sodium lauryl sulfate, sodium benzoate, sodium saccharin, menthol, and distilled water. The weighed ingredients were then combined in a mixer until homogenous. The aroma was subsequently added to enhance the sensory appeal, and the resulting formulation was packed into tube containers [14,15].

Plaque Index Examination

Thirty children were randomly selected and divided into three groups: a control group receiving toothpaste with 0% *T. cacao* seeds extract and two experimental groups receiving toothpaste with 1% and 2% *T. cacao* seeds extract, respectively. Prior to the intervention, the plaque index of each child was assessed using a standardized scoring system. Following the intervention, the plaque index was reassessed to evaluate any changes. Data obtained were analyzed to compare changes in plaque accumulation within each group and to assess potential differences between the groups receiving different concentrations of *T. cacao* seed extract. The study adhered to ethical principles, with informed consent obtained from parents or legal guardians and confidentiality of participants' data maintained throughout the study.

Statistical Analysis

Plaque indices were presented as mean and median. Levene test was carried out to identify whether the data were homogenous. Statistical difference was estimated by Analysis of Variance (ANOVA) followed by Tukey post hoc. All statistical analysis was carried out using SPSS Version 20 [16].

Results and Discussion

Secondary metabolites of *T. cacao* seeds

The maceration of *T. cacao* seeds using methanol produced 61.78-gram extract from a total of 2 kg sample; hence, the total yield was 3.089%. Results from the qualitative analysis of the secondary metabolites contained in the methanolic extract of *T. cacao* seeds are presented in Table 1. The analysis revealed that the extract is positive, containing phenolics, tannins, flavonoids, terpenoids, saponins, and alkaloids. Steroids were not observable during the test. The photographed images of the sample before and after the addition of specific reagents are presented in Figure 1.

Table 1. Results from the phytochemical testing.

Secondary metabolites	Observation	Remarks
Phenolics	Formation of green color	+
Tannins	Formation of cloudy white color	+
Flavonoids	Formation of red/purple color	+
Steroids	No formation of green/blue color	-
Terpenoids	Formation of red color	+
Saponins	Formation of stable foam	+
Alkaloids		
Dragendorff	Formation of brick red color	+
Mayer	Formation white precipitate	+
Wakner	Formation of brown precipitate	+

Presence (+), absence (-)



Figure 1. Photographed images of *T. cacao* seeds extract before and after the phytochemical test.

Effects on the Plaque Index

Based on the Levene test, presented in Table 2, the plaque indices assessed before the intervention were considered homogenous ($P > 0.05$). However, the results of the Levene test on data obtained after the intervention suggest heterogeneity with $P < 0.01$. The data were then analyzed using ANOVA, where the results are presented in Table 3. Differences in plaque indices among the three groups were observed to be statistically significant, as indicated by $P = 0.21$, and supported by a high value of $F (4.449)$. Following the intervention, plaque indices of children receiving toothpaste containing 1% *T. cacao* seeds extract are significantly lower than control (mean difference: 2.060 [95%CI: 0.63—3.49]; $P = 0.006$) (Table 4). Nonetheless, no significant difference between the control and 1% *T. cacao* seeds extract group (mean difference: 1.260 [95%CI: -0.17—2.69]; $P = 0.082$). Plaque indices of children in 1% and 2% *T. cacao* seeds extract groups were not significantly different either (mean difference: -0.800 [95%CI: -2.23—0.63]; $P = 0.261$) (Table 4). The toothpaste formulation result can be seen in Figure 2.

Table 2. Results from Levene test for homogeneity.

Test Type	Before Intervention				After Intervention			
	Levene Statistic	df1	df2	P	Levene Statistic	df1	df2	P
Based on Mean	1.352	2	27	.276	11.631	2	27	.000*
Based on Median	0.923	2	27	.409	9.477	2	27	.001*
Based on the Median and with adjusted df	0.923	2	17.411	.416	9.477	2	16.151	.002*
Based on trimmed mean	1.232	2	27	.307	12.043	2	27	.000*

*Statistically significant at $P < 0.05$

Table 3. Results from ANOVA of plaque indices after the intervention.

Comparison	Sum of squares	df	Mean square	F	P
Between Groups	21.571	2	10.785	4.449	.021*
Within Groups	65.459	27	2.424		

*Statistically significant at $P < 0.05$

Table 4. Results from ANOVA post hoc analysis on plaque indices after the intervention.

Comparison	Mean difference (95% CI)	Std. Error	P
0% versus 1% extract	2.060 (0.63—3.49)	0.696	.006*
0% versus 2% extract	1.260 (-0.17—2.69)	0.696	.082
1% versus 2% extract	-0.800 (-2.23—0.63)	0.696	.261

0% extract is the control group
*Statistically significant at $P < 0.01$

**Figure 2.** Toothpaste formulation result.

Discussion

Herein, a total extraction yield was found to be 3.089%. The yield value serves as a crucial indicator for assessing the effectiveness of research interventions. A higher yield value signifies greater effectiveness of the extraction process. The *T. cacao* extract was then found to contain phenolics, tannins, flavonoids, terpenoids, saponins, and alkaloids. Extraction time and type of solvents used during the macerations, among other parameters, determine the quantity and secondary metabolites drawn from the sample [14,15]. The rationale behind utilizing methanol as a solvent lies in its capability to attract polar active secondary metabolites [17,18]. It is suggested that the compounds present in the methanol extract of cocoa beans are predominantly polar, including flavonoids, glycosides, tannins, and alkaloids [18]. Apart from the extraction process, the composition of secondary metabolites found in plants is influenced by various internal and external factors. Internal factors, such as the influence of varieties/genes, include external factors, namely the influence of sunlight, rainfall, soil structure, and climate in the area, so there are differences in the active substance content of *T. cacao* seeds. The harsh environmental conditions of Sare Aceh and the location from which the sample originates significantly influence both the percentage yield and the weight of the extract, given the remarkable adaptability of this type of plant.

Alkaloids possess antibacterial properties by disrupting the peptidoglycan constituents within bacterial cells, inhibiting complete cell wall formation, and inducing cell death. Flavonoids act as antibacterials by complexing extracellular proteins, leading to structural damage and alterations in bacterial cell membrane functionality. Antibacterial mechanisms of the aforementioned compounds involve their complexation with extracellular and soluble proteins, resulting in membrane damage and subsequent release of intracellular contents [19]. Tannins are thought to constrict cell walls or membranes, disrupting cellular permeability and impeding essential cellular functions, ultimately hindering growth or inducing cell death [19]. The antibacterial mechanism of tannins involves inhibiting enzymes like reverse transcriptase and DNA topoisomerase, thereby preventing bacterial cell formation. Additionally, phenolic

compounds exert antimicrobial effects by damaging microbial plasma membrane lipids, causing leakage of cellular contents [19].

Findings from the present study also suggest that toothpaste added with *T. cacao* seeds extract significantly improved its effect in elevating dental plaque, as indicated by plaque index. This indicates that *T. cacao* seed extract is an essential toothpaste additive that can help reduce plaque accumulation, thereby preventing dental caries and periodontal complications such as gingivitis. Such an effect might be associated with the antibacterial properties of the extract as the plaque biofilm is predominantly produced by *S. mutans* and other bacteria in the oral cavity. This is corroborated by the fact that the extract contains various secondary metabolite compounds, including alkaloids, steroids, terpenoids, saponins, flavonoids, and phenolics. As previously reported, flavonoids, saponins, and alkaloids contribute to the potent antibacterial activity of the extract against *S. mutans*. These compounds exhibit bacteriostatic properties, inhibiting the growth of *S. mutans* bacteria and thereby contributing to plaque reduction [20,21].

It is worth noting that conventional non-herbal toothpaste is purported to reduce the plaque index primarily due to the presence of fluoride, chlorhexidine, and triclosan, renowned for their antibacterial properties. However, fluoride content in toothpaste may not be suitable for children, as excessive fluoride intake poses risks such as fluorosis, toxicity, and tooth demineralization. Attempts to enhance the antibacterial efficacy of toothpaste by increasing fluoride and antibacterial ingredients like triclosan may exacerbate these risks. Consequently, regulatory bodies such as the Food and Drug Supervisory Agency have issued directives to withdraw toothpaste products intended for children containing fluoride concentrations exceeding 500 ppm, emphasizing the imperative of caution in formulating dental products tailored for pediatric use.

Limitations of the Study and Further Research

Despite the promising findings, several limitations warrant consideration. Firstly, the small sample size of the study may limit the generalizability of the results to broader populations. Additionally, the relatively short duration of the intervention period may not capture the long-term effects of *T. cacao* seed extract-added toothpaste on plaque reduction and oral health outcomes. Moreover, variations in individual oral hygiene practices, dietary habits, and compliance with the intervention may have influenced the observed outcomes. Furthermore, the study's design, which focused on healthy children aged 8-10 years, may not fully capture the efficacy of *T. cacao* seed extract in populations with diverse oral health conditions or age groups. Addressing these limitations in future research endeavors will provide a more comprehensive understanding of the efficacy and safety profile of *T. cacao* seed extract in oral care products. To address the limitations identified in this study and advance our understanding of *T. cacao* seed extract in oral care, further research is warranted. Future studies could involve larger sample sizes encompassing diverse populations to enhance the generalizability of the findings. Longitudinal studies with extended intervention periods are essential to assess the sustained effects of *T. cacao* seed extract-added toothpaste on plaque reduction and overall oral health. Additionally, investigations into the optimal concentration of *T. cacao* seed extract, formulation compatibility with other oral care ingredients, and potential adverse effects are crucial for the development of safe and effective oral care products. Furthermore, exploring the mechanisms underlying the antibacterial and plaque-reducing effects of *T. cacao* seed extract at the molecular level will provide valuable insights into its mode of action and therapeutic potential in oral health management.

Conclusions

In conclusion, the investigation into the efficacy of *T. cacao*-added-based toothpaste in reducing plaque index among children has shown promising results, suggesting a potential role for the extract as a viable additive in oral care products aimed at improving oral hygiene. Phytochemical analysis of the methanol extract from *T. cacao* seed revealed the presence of various secondary metabolites, including phenolics, tannins, flavonoids, terpenoids, saponins, and alkaloids, which are thought to contribute to the reduction of plaque by inhibiting bacterial growth. However, to fully elucidate the efficacy and safety profile of these compounds, further research with better-designed studies, larger populations, and longer-term follow-ups is recommended. Additionally, comprehensive safety assessments are essential to ensure the development of dental products incorporating cocoa bean extract that are both effective and safe for widespread use in oral hygiene routines.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

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Conflicts of Interest: All the authors declare that there are no conflicts of interest.

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